Fertility in Mice after Prenatal Exposure to Benzo[*a*]pyrene and Inorganic Lead

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Experimental evidence suggests that inorganic lead and benzo[*a*]pyrene (BaP) suppress the development of primordial oocytes during fetal life. We examined the single and combined effects of prenatal exposure to BaP and moderate doses of lead. The fertility and ovarian morphology of F_1 female NMRI mice in four treatment groups (nine mice per group) were investigated: control; lead (F_0 given 1 g $PbCl_2$/L in drinking water until mating); BaP (10 mg/kg body weight daily by oral intubation on days 7–16 of F_0 pregnancy); and combined lead and BaP. F_1 groups exposed prenatally to BaP either alone or in combination with inorganic lead showed markedly reduced fertility with few ovarian follicles compared to controls, whereas the group exposed to lead only had measures comparable to the controls. Mice exposed to both lead and BaP had a significantly longer gestation period (days to litter) compared to mice exposed only to BaP, lead, or controls. There is a nonsignificant indication that the compounds together further reduce number of offspring, number of litters, and litter size. These results suggest that lead and BaP have synergistic effects on impairment of fertility. The possibility of synergism may be of human relevance as inorganic lead and BaP are ubiquitous environmental pollutants. *Key words*: benzo[*a*]pyrene, fertility, inorganic lead, oogenesis, synergism. *Environ Health Perspect* 103:588–590 (1995)

The period of development of the female gonads in fetal life may be critical for fertility in adult life. Rodents show a reduced number of primordial oocytes or reduced fertility after prenatal exposure to inorganic lead (*1–3*), benzo[*a*]pyrene (BaP) (*4*), and other agents (*5,6*). Inorganic lead is also a suspected developmental neurotoxicant (*7*) and could possibly have adverse effects on the developing reproductive system through action on the hypothalamic-pituitary axis during fetal life. Studies exploring if similar mechanisms may be responsible for human subfecundity are scarce; in an epidemiologic study maternal smoking in the pregnancy was associated with reduced fecundability among their daughters in later life (*8*).

The reproductive effects of environmental pollutants are currently receiving attention, due in part to the possibility of human infertility (*9*). BaP and inorganic lead are of special interest. Both are ubiquitous environmental pollutants, and BaP is a component of cigarette smoke. We therefore conducted an experiment exposing mice prenatally to inorganic lead and BaP. The purpose of the study was to investigate lead and BaP for synergistic effects on female fertility. We chose a treatment dose of lead that was judged from the blood lead levels to be comparable to exposure levels found in many occupational settings. The treatment dose of BaP was similar to the lowest dose that induced subfertility in an earlier report (*4*).

Methods

Lead(II) chloride (CAS no. 7758-95-4) was supplied from Baker (Deventer, Holland). Benzo[*a*]pyrene (CAS no. 50-32-8) was a product of Sigma Chemical Company (St. Louis, Missouri).

Male and F_0 female Bom:NMRI mice were acclimatized to a 12/12 hr light/dark cycle (lights on at 0600 hr) at 24 ± 1°C. Laboratory chow (EWOS, R34) and tap water were provided *ad libitum*. Males were individually caged except during mating. We placed F_0 females in groups of three until the last week of pregnancy when they were caged alone.

At the age of 9 weeks, F_0 females were randomly assigned to one of four treatment groups, nine mice per group. Two groups received tap water with 1 g $PbCl_2$/L(0.75 g Pb) during the 6 weeks before mating, whereas the remaining two groups received tap water without any additions. We discontinued the lead treatment before mating to avoid exposure of the males. All F_0 females were caged with sexually active males. Females were inspected twice daily for a vaginal plug, which, in case of pregnancy, was counted as day 0 of gestation. During days 7–16 of pregnancy, one of the tap water groups and one of the lead–water groups received a daily treatment of 0.2 mL corn oil BaP (10 mg/kg body weight by oral intubation), and the remaining two groups received corn oil. Thus, the four treatment groups, each comprising nine F_0 females, were the control group, the lead group, the BaP group, and the lead plus BaP group. We recorded weights and sampled blood for lead measurement from tail veins on the day before caging with the males (Table 1). Assessed from a pilot trial on female mice of the same strain, the blood lead values in the lead-treated groups should give values of approximately 2 µmol/L 10 days later (during the second week of pregnancy). No F_0 females showed signs of general toxicity, and they all proved fertile. We kept F_0 females with their offspring until after weaning (21 days after delivery).

One F_1 female from each of the 36 litters was allocated in the experiment, each belonging to one of the four exposure groups they had been assigned to *in utero*. At the age of 6 weeks, each F_1 female was caged for 6 months with an untreated male proven to be sexually active. Males for four females that did not become pregnant after 30 days were replaced, but this procedure did not lead to any pregnancies. Dates and sizes of litters were recorded. F_2 offspring were inspected for gross deformities at birth, and their weight and sex were recorded at day 2 after birth when they were killed by cervical dislocation.

After 6 months of continuous breeding, the F_1 females were euthanized; the right ovary was excised, trimmed, and weighed. Ovaries were fixed in buffered formalin, embedded in paraffin, and 3-µm parasagittal sections were prepared. Three slides, made from tissue 90 µm apart, were stained with hematoxylin-azophloxine-saffron and reticulin and examinated by light microscopy.

The F_1 female was taken as the basic unit of experimentation. Several measures of fertility and ovarian morphology were recorded without knowledge of the treatment group. Measures of fertility for each F_1 female were number of offspring, number of litters during the breeding period, median litter size, and median days between deliveries (with the start of the experiment as the starting point for the first interval).

We recorded the weight of the right ovary for each F_1 female and counted follicles and corpora lutea in the three histological sections. Follicles were classified according to Pedersen and Peters (*10*).

Treatment effects on fertility, ovarian weights, and counts in the microscopic examination were evaluated by nonparametric tests (Wilcoxon rank sum test, Kruskall-Wallis test). Significance levels of <0.05 (two-tailed) were taken as criterion of treatment effects. The statistical analyses were performed with the BMDP software package (*11*).

Results

The 36 mated F_1 females gave birth to a total of 1985 offspring distributed over 182 litters. The distribution over the treat-

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Table 1. Descriptive values (median, range) for F_0 and F_1 female mice in the four treatment groups

	F_0 females (n = 9/group)				F_1 females (n = 9/group)	
	Body weight at time of mating (g)		Blood lead at time of mating (μmol/L)		Body weight after first pregnancy (g)	
Treatment group	Median	Range	Median	Range	Median	Range
Control	37.8	34.6–41.4	0.04	0.01–0.10	38.7	37.3–43.9
Lead	36.5	33.3–43.9	3.37	2.44–3.80	39.8	37.9–44.7
BaP	35.9	32.8–40.3	0.04	0.02–0.08	37.1	34.1–44.3
Lead plus BaP	37.9	32.7–46.3	3.71	2.70–5.26	38.0	32.9–44.7
Heterogeneity between groups, χ^2 (p)[a]	0.93 (0.82)		27.3 (<0.0001)		1.98 (0.58)	

BaP, benzo[*a*]pyrene.
[a]Chi-square with 3 degrees of freedom; two-tailed p-value.

Table 2. Values of different measures of fertility over the four treatment groups among F_1 female mice

	Treatment group				Heterogeneity between groups, χ^2 (p)[a]
Effect	Control (n = 9)	Lead (n = 9)	BaP (n = 9)	Lead plus BaP (n = 9)	
No. of litters	67	72	29	14	
No. of offspring (stillborn)	785 (4)	860 (8)	248 (8)	92 (9)	
Median no. offspring/F_1 (range)	92 (26–121)	95 (50–122)	22** (0–86)	4** (0–48)	24.5 (<0.0001)
Median no. litters/F_1 (range)	8 (3–8)	8 (8–8)	3** (0–8)	2**(0–5)	22.8 (<0.0001)
Median litter size/F_1 (range)	11.5 (6–15)	13 (6–18)	8** (3–11)	6.5** (1–12)	10.6 (0.01)
Median no. days between litters/F_1 (range)	20.5 (20–21)	20.5 (20–21)	21* (20–23)	22.5** (21–29)	15.5 (0.001)

BaP, benzo[*a*]pyrene.
[a]Chi-square with 3 degrees of freedom; two-tailed p-value.
*Significantly different from control group (two-tailed $p < 0.05$).
**Significantly different from control group (two-tailed $p < 0.005$).

ment groups is given in Table 2. The lead group had litters and offspring numbers that were slightly higher than the control group, whereas the BaP group and the lead plus BaP group both had considerably lower fertility as judged by these measures. The cumulative number of F_2 offspring over time and the distribution of number of litters among F_1 females in the four treatment groups are illustrated in Figures 1 and 2, respectively. Four F_1 females (one BaP treated, three lead plus BaP treated) were infertile in the study; only 1 out of 18 F_1 females producing more than 6 litters belonged to either of the 2 groups receiving BaP (Fig. 2).

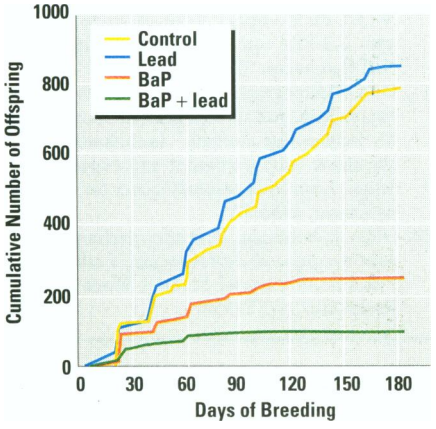

Figure 1. Cumulative number of F_2 offspring recorded over 6 months for the four F_1 treatment groups.

The Kruskall-Wallis test demonstrated highly significant heterogeneity over the treatment group for all measures of fertility: number of offspring, number of litters, median number of offspring per litter, and days between litters (Table 2). This was due to markedly lower fertility measures in the BaP and the lead plus BaP groups compared to the control group, whereas the lead group performed nonsignificantly better than the control group for all measures. In the comparison between the BaP group and the lead plus BaP group, the latter showed poorer fertility for all measures. Some of those differences were large but were only significant for median days between litters (Z-value = 1.98, two-tailed p = 0.05). For other effect measures, the significance levels ranged from 0.09 to 0.32.

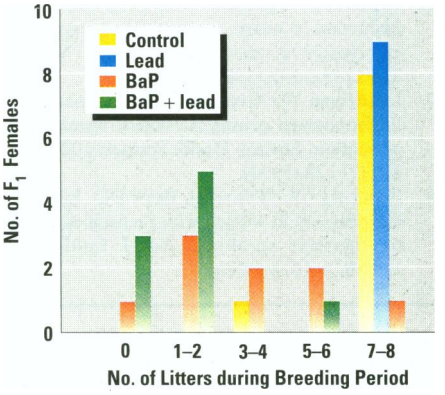

Figure 2. Frequency distribution of total litters recorded over 6 months among F_1 females in the four treatment groups.

A similar pattern was evident from examination of the ovaries (Table 3). Ovary weights, counts of follicles, and counts of corpora lutea were different in the treatment groups, with low weights and depletion of follicles and corpora lutea for most animals in the BaP and lead plus BaP groups. The F_1 females in the lead group had nonsignificantly higher weights and counts of follicles and corpora lutea compared to the control group. The lead plus BaP group had lower counts of follicles and corpora lutea and slightly higher median ovarian weight than the BaP group, but all differences were nonsignificant in the Wilcoxon rank sum test.

Discussion

We have shown that prenatal administration of 10 mg BaP/kg maternal body weight (with or without lead) leads to subfertility and a marked reduction in the number of ovarian follicles in F_1 females. These results are in agreement with a previous report (*4*) and are probably due to impaired development of primordial oocytes. This interpretation is further supported by an earlier report on toxic effects of BaP on primordial oocytes in weanling mice (*12*).

No treatment effects of lead were detected in the study. This is not surprising since we used moderate doses resulting in maternal blood lead levels about 2 μmol/L during the second week of pregnancy; this is considered to be the critical time of fetal folliculogenesis (*3*). This dose was far lower than that reported to have an independent effect on primordial germ cells (*3*) and fertility (*1–3*).

To our knowledge, the combined effect on fertility of inorganic lead and BaP has not been reported earlier. Our study was designed to investigate the hypothesis of an interaction between the two agents. We found a synergistic action of inorganic lead on the BaP effects for days between litters, and the manifestations of combined lead and BaP treatment were stronger, but not

Table 3. Values of different measures of ovarian effects over the four treatment groups among F_1 female mice[a]

Effect	Median (range) Control (n = 9)	Lead (n = 7)	BaP (n = 8)	Lead plus BaP (n = 8)	Heterogeneity between groups, χ^2 (p)[b]
Ovarian weight (mg)	13 (13–20)	14 (11–25)	9* (7–13)	10 (2–17)	12.4 (0.006)
No. of small follicles/F_1	44 (1–137)	60.5 (15–150)	0**(0–68)	0**(0–35)	18.8 (0.0003)
No. of medium follicles/F_1	9 (5–25)	12.5 (2–30)	0** (0–57)	0** (0–2)	19.3 (0.0002)
No. of large follicles/F_1	14 (6–23)	21.5 (12–29)	0** (0–19)	0** (0-0)	23.6 (0.0001)
No. of corpora lutea/F_1	16 (6–35)	22 (15–57)	0** (0–14)	0** (0–4)	23.4 (<0.0001)

BaP, benzo[*a*]pyrene.
[a]Histologic examinations were performed on three sections of the right ovary. Results were discarded for two animals in the lead group and one animal each in the BaP and the BaP plus lead-treated groups.
[b]Chi-square with 3 degrees of freedom; two-tailed *p*-value.
*Significantly different from control group (two-tailed $p < 0.05$).
**Significantly different from control group (two-tailed $p < 0.005$).

significantly so, for almost all indicators of impaired follicular development and fertility compared to the BaP treatment alone. It may be relevant that some lead compounds have synergistic effects on model carcinogens in rodents (*13–16*).

Mechanistic interpretations on the synergism between lead and BaP on the basis of our results can only be speculative. BaP seems to have a direct effect on primordial oocytes (*4,7*). Fetal treatment with high doses of inorganic lead also decreases the number of primordial follicles, possibly as an effect on the migration or multiplication of the developing germ cells (*2*). Lead is also a developmental neurotoxicant, and its synergistic action might be explained by a disturbance of the neuroendocrine balance that alone was not sufficient to impair fertility. However, Wide (*2*) found only small and nonsignificant alterations in the levels of ovarian steroid hormones for mice exposed prenatally to lead in doses that clearly reduced the number of primordial follicles.

The lead and BaP doses chosen in our study were probably not optimal. The lead dose was comparable to human exposures, and the daily BaP dose in our study was equal to the lowest effect level in an earlier study, but its effect on fertility was strong (*4*). The daily dose (10 mg/kg) is about 1 million times the main stream dose in 100 cigarettes (*17*). Fertility as outcome might have been more sensitive to the combined effects of lead and BaP if the BaP dose had been lower. BaP alone had a profound effect; even if the lead plus BaP group had almost total depletion of follicles and several animals were infertile, the differences were not significant.

There is currently much concern about human fertility (*9*). Biology indicates that the prenatal development of primordial germ cells may be crucial. Exposures to common environmental xenobiotics are under suspicion of interfering with gonadal development (*9*). Human exposures from environmental sources are considerably lower than exposures producing effects in laboratory animals but could be more relevant in case of synergistic actions. Human studies addressing effects of environmental agents are warranted, but the needed multigenerational designs restrict the possibilities (*18*). Animal models that have been developed should be further applied.

References

1. Stowe HD, Goyer RA. The reproductive ability and progeny of lead-toxic rats. Fertil Steril 22:755–760 (1971).
2. Wide M. Lead exposure on critical days of fetal life affects fertility in the female mouse. Teratology 32:375–380 (1985).
3. Wide M, d'Argy R. Effect of inorganic lead on the primordial germ cells in the mouse embryo. Teratology 34:207–212 (1986).
4. MacKenzie KM, Angevine DM. Infertility in mice exposed *in utero* to benzo[a]pyrene. Biol Reprod 24:183–191 (1981).
5. Tam PPL, Snow MHL. Proliferation and migration of primordial germ cells during compensatory growth in mouse embryos. J Embryol Exp Morphol 64:133–147 (1981).
6. Chen Y-T, Mattison DR, Feigenbaum L. Reduction in oocyte number following prenatal exposure to a diet high in galactose. Science 214:1145–1147 (1981).
7. Landrigan PJ, Graham DG, Thomas RD. Environmental neurotoxic illness: research for prevention. Environ Health Perspect 102(suppl 2):117–120 (1994).
8. Weinberg CR, Wilcox AJ, Baird DD. Reduced fecundability in women with prenatal exposure to cigarette smoking. Am J Epidemiol 129:1072–1078 (1989).
9. Michal F, Grigor KM, Negro-Vilar A, Skakkebæk NE. Impact of the environment on reproductive health: executive summary. Environ Health Perspect 101(suppl 2): 159–167 (1993).
10. Pedersen T, Peters H. Proposal for a classification of oocytes and follicles in the mouse ovary. J Reprod Fertil 17:555–557 (1968).
11. Dixon WJ, Brown MB, Engelman L, Jennrich RI. BMDP statistical software manual. Berkeley CA:University of California Press, 1990.
12. Mattison DR, Thorgeirsson SS. Smoking and industrial pollution, and their effects on menopause and ovarian cancer. Lancet 1:187–188 (1978).
13. Hinton DE, Lipsky MM, Heatfield BM, Trump BF. Opposite effects of lead on chemical carcinogenesis in kidney and liver of rats. Bull Environ Contam Toxicol 23:464–469 (1979).
14. Hiasa Y, Ohshima M, Kitahori Y, Fujita T, Yuasa T, Miyashiro A. Basic lead acetate: promoting effect on the development of renal tubular cell tumors in rats treated with *N*-ethyl-*N*-hydroxyethylnitrosamine. J Natl Cancer Inst 70:761–765 (1983).
15. Tanner DC, Lipsky MM. Effect of lead acetate on *N*-(4'-fluoro-4-biphenyl)acetamide-induced renal carcinogenesis in the rat. Carcinogenesis 5:1109–1113 (1984).
16. Shirai T, Ohshima M, Masuda A, Tamano S, Ito N. Promotion of 2-(ethyl-nitrosamino)ethanol-induced renal carcinogenesis in rats by nephrotoxic compounds: positive responses with folic acid, basic lead acetate, and *N*-(3,5-dichlorophenyl)succinimide but not with 2,3-dibromo-1-propanol phosphate. J Natl Cancer Inst 72:477–482 (1984).
17. IARC. Monographs on the evaluation of the carcinogenic risk of chemicals to humans, vol 32. Polynuclear aromatic compounds, part I. Chemical, environmental and experimental design. Lyon:International Agency for Research on Cancer, 1983;37.
18. Olsen J. Is human fecundity declining—and does occupational exposures play a role in such a decline if it exists? Scand J Work Environ Health 20:72–77 (1994).